DIGESTIVE ACTIVITY OF MESENCHYME AND ITS DERIVATIVES.

II. Proteins as Object (A. EDESTIN.)

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1. STATEMENT OF THE PROBLEM.

The recently obtained results regarding the digestive activity of the adult splenic cells of the fowl upon two mammalian tumors, the Ehrlich sarcoma and the Crocker Fund tumor 180, have raised a series of problems. Owing to the complexity of the conditions involved in the experiments referred to, they could not be easily attacked and solved at that time.

Has the embryonic mesenchyme, found to be powerless against the proliferation of the two heterogenous tumors in the fowl, no power at all to digest foreign proteins in dead or living form? Is only the adult mesenchyme of the spleen to be regarded as a tissue endowed with a specific digestive function, or can any mesenchymal cell and possibly some of its derivatives, wherever found in the organism, exhibit under definite conditions one of the most fundamental powers of living matter, *i.e.*, that of digesting particulate proteins? Finally, the act of digestion performed by a group of cells, in a well-defined region of the organism, is it to be regarded as a purely local phenomenon, or as a process, the effects of which extend beyond the boundaries of the tissue directly involved?

These questions can be answered only in a fragmentary and insufficient way on the basis of already known data. Embryonic mesenchymal cells were occasionally observed to exercise a phagocytic and digestive action upon dead or weakened cells, but little is known as to whether they possess a similar power against foreign protein. Other than splenic mesenchymal cells

have occasionally been observed in the adult to succeed in completely digesting heterogenous proteins, as for example, catgut of mammalian origin which is digested by the stroma cells of different animals. Though suggestive of a widespread digestive power of the mesenchyme, these observations do not allow any definite conclusions regarding its extent and its various aspects. Finally, the question has hardly been raised, as to whether a digestive activity temporarily developed by the mesenchyme or by its derivatives in a definite region, might not have a general effect upon the whole organism of the animal under experiment. In this connection, the production of induced immunity against tumors by introduction into the organism of particles of tissue of the same species as that bearing the tumor and the immunization against pathogenic bacteria by introducing the same organisms in a weakened state, is of interest. As to how a local process displayed in a well-defined region of the organism can influence its general properties and through what mechanism this can be effected, only few hypotheses and even less data can be found.

In order to approach the problems stated above, different heterogenous, non-injurious proteins should be introduced among stroma cells other than those of the spleen. The study of their digestion, especially when excessive amounts are introduced, would throw light upon the question as to whether easily mobilizable cells from remote parts of the organism will participate in this process along with the cells found in close proximity to the injected masses. The study of the digestion of the substances introduced should be pursued not only until its completion, but special care should be given to the fate of the phagocytes, and a method should be devised for identifying them in any part of the organism which they might enter, either in a passive way or by their own active movements. Rather small animals only can be used, the size of which would not offer insurmountable difficulties for the study of the final distribution of these phagocytes.

A favorable object for such studies has been found in the tadpole. Various amounts of different heterogenous proteins, insoluble in saline, such as edestin, fibrin, coagulated egg albumin, were introduced into the transparent tail. When it was found that

enormous amounts of introduced material were easily digested within the tadpole tail, the details of this process and especially the structure and origin of those cells which exercised this activity were determined. When it was found that the region into which the material was introduced and which was promptly invaded after the experiment by numberless cells was as promptly abandoned by most of them, an attempt was made to determine their new whereabouts and to identify them. This part of the work has so far been only partly successful. The ultimate distribution of the phagocytes will be studied further in greater detail and the results given in another paper.

Material and Method Used.

The transparent tail of the tadpole has been frequently used not only as a convenient object for observing normal processes of growth and differentiation, but also as a medium in which the activity of the various stroma cells, under experimental conditions, could be studied with remarkable ease. Work on the tadpole tail has been done in this country chiefly by Eliot R. Clark and Eleanor Linton Clark. They studied in it the growth of vessels in vivo. They introduced under its epidermis microscopic particles of paraffin, India ink, croton oil and starch granules and studied the reaction called forth in the adjacent tissues by the presence of these foreign materials. Some of the materials used by them (paraffin and India ink) were of such a nature as would lead us to expect only a physical reaction from the adjacent cells. Among the other material used by them, the croton oil produced upon the adjacent tissue injurious effects such as it would upon any other living tissue. Uncooked starch granules produced no other effect than foreign bodies, while starch granules cooked to the point of gelatinization proved to be a powerful chemotactic agent for leucocytes. Ingestion of such starch granules took place, but digestion of it was not followed.

The result of this work did not permit of any decisive conclusions regarding the digestive activity of the tadpole stroma for the simple reason that most of the substances used are not digestible. If the same material were introduced into our regu-

lar digestive tract, no other results would be obtained, than those described by the authors in relation to the stroma cells of the tadpole. Paraffin would produce no direct effect upon the lining of the digestive tube, some of the granules of India ink might be incorporated by cells, if a sufficiently long contact could have been secured. Croton oil would produce an intensive inlammation, aseptic if applied by itself in a milieu free of bacteria. Starch granules would be digested, but even these only in certain parts of the digestive tract.

Our experiments consist of introducing various amounts of suspensions of different protein substances into the thin edges of the tadpole tail. The injections were made with extremely fine glass pipettes. After numerous more or less unsuccessful attempts to use rather complex and apparently well-devised methods of injecting, recourse was taken to the simple and efficacious way used by Doctor Clark and courteously demonstrated by him. The substances, suspended in saline solution, were blown into the tissue by lung pressure. The injections were made both into the dorsal and ventral edges of the tail. The epidermis of the tail is easily pierced in the caudal part of its edge by the fine end of a glass pipette containing a thick suspension of a fine pulverized protein, between its dorsal border and the axial strand of the denser tissue. The pipette is then pushed at about equal distance from dorsal and ventral borders of the transparent plate for about 2–4 mm. in the cranial direction. The other end of the pipette is connected with a rubber tube. The substance is introduced into the tissue of the tadpole by blowing into the tube, considerable effort being required in order to force the substance through the glass capillary. It is essential, while blowing, to gradually withdraw the glass pipette from under the skin.

Since large amounts, as well as a few particles of various substances were introduced, medium and large-sized tadpoles were chosen for the experiment. A successful injection could be easily determined, the material injected appearing under the skin as an opaque streak. Though in some cases the substances were introduced in excessive amounts (the strand of injected substance appearing to be over 3 mm. in length and to occupy half

of the thickness of the fin) the animals did not show uneasiness from the effect of the operation. Hemorrhages could not be avoided during injection, but those specimens in which hemorrhage could be macroscopically discerned were eliminated. Observations in vivo were occasionally made and a large number of fixed tadpoles at different stages after injection were secured. The animals were allowed to live from 2 hours to 3 or 4 weeks after the injection.

The first set of experiments, of which a report is given in the present paper, consisted in injecting various amounts of edestin. This substance, insoluble in water, appears macroscopically in the form of minute particles, irregular in shape, rather uniform and rarely exceeding in size the eosinophilic granules of leucocytes. Every edestin granule ingested by cells can easily be identified in an eozin-azur preparation after Zenker-formol fixation, the granules staining a brilliant red.

We wish to express to Professor Gies our great indebtedness for chemically purifying this substance.

Structure of the Dorsal Fin of a Tadpole Tail.—A short description of the structure of the edge of the tadpole tail is necessary in order to know what kind of cells will be in immediate contact with the substance introduced into it. It consists of a plate of loose mesenchyme covered on both its surfaces by epidermis. The epidermis contains three differently organized layers of cells, all of them exhibiting frequent mitoses. The superficial layer of cuboidal epithelial cells is covered by a thin cuticular border. The basal layer of epithelial cells consists of characteristic cuboidal and columnar cells containing sharply defined filaments. The middle layer of the epidermis consists of pigmented cells with peculiarly incurved nuclei. Chromatophores in the form of many branched cells are not infrequently found in this layer. The epidermis is lined by a heavy basal membrane. Numerous white fibers traverse the tissue between the two basal membranes.

The loose tissue of the plate contains scattered mesenchymal cells and vessels, a few strands of smooth muscle tissue and nerves. No wandering cells of any kind are normally present in this

tissue. A continuous layer of mesenchymal cells is situated directly under the basal membrane of the epidermis. These cells, as well as those situated within the plate itself, are typical loose connective tissue cells with numerous long cytoplasmic processes, with oval nuclei usually containing well-defined nucleoli and minute particles of chromatin. Occasional mitoses are found among them. Chromatophores are few in this tissue, but are not infrequently found surrounding the vessels and accompanying even tiny vascular branches.

Results of the Experiments.

In describing and analyzing the various phases of the reaction developed around the injected material, a distinction has to be made between the processes due to the injury proper and those which develop in consequence of the presence of foreign matter, digestible particulate protein in this case. The reaction due to the injury is exhibited partly by local cells, partly by elements brought in by the blood current from remote parts of the organism. The reaction due to the injury is, in these experiments, only slight in both of these aspects and is greatly overshadowed, shortly after the injection, by processes depending upon the presence of the injected material.

Effect Produced by the Injury during Injection.—Besides a slight injury of the epidermis, a few cells of which are sometimes carried into the mesenchymal plate, the direct consequence of the injection is the boring of a canal in the midst of the loose mesenchymal tissue and the filling of it by finely pulverized proteinic matter. It is remarkable how seldom appreciable hemorrhages are produced, and only very few blood corpuscles are regularly found free in the intercellular spaces as a result of the puncture of a few small vessels. By pushing the glass capillary under the epidermis the tissue is simply compressed, this compression being further exercised by the thick suspension of edestin after the withdrawal of the glass capillary. Microscopically the injected material appears in the form of a rather sharply defined strand of densely aggregated particles. The immediately adjacent tissue becomes slightly edematous and the white fibers traversing the fin plate

swollen. Only few mesenchymal cells are found in direct contact with the edestin particles fifteen minutes after the injection, but even at that time these cells, if compared with those situated at a distance from the injected material, seem to be larger, their processes shorter and plumper and their cytoplasm often filled with tiny vacuoles giving it a foamy appearance. There is no doubt but that these changes in the mesenchymal cells, observed almost immediately after the injection, are not to be regarded as a specific response to the introduction of the edestin. Practically the same changes are seen after an injury produced by the introduction of the glass pipette without injection.

A breaking off of the syncytial arrangement of the cells and formation of typical wandering cells can be observed in those few mesenchymal cells which are in close proximity to the injured tissue. The number of wandering cells formed in loco at the expense of the mesenchymal cells is small indeed, the mesenchymal cells themselves being scarce. These local changes are slow and are quickly overshadowed by the appearance of cellular elements brought in by the blood stream.

Effect Produced by the Presence of the Injected Substance.—The changes observed in the region in which the injected masses are situated and dependent upon their presence are in part identical to those found, if only injury with the glass pipette were produced and the injection omitted. They differ greatly, however, in their intensity and in their duration. In describing the changes observed around and within the injected mass, three different phases will be reported under separate sections. (1) Appearance of wandering cells. (2) Digestive processes. (3) Phagocytes after digestion.

1. *Appearance of Wandering Cells.*—As mentioned above, vessels are seldom ruptured during injection and only a few groups of extravasated blood cells are found around the injected material. Among those the white blood corpuscles are seen to exhibit an intensive activity, the erythrocytes, however, lie inert in the intercellular spaces at first. Shortly after the injection the white blood corpuscles become greatly increased in numbers around the injected masses. Not only in nearest proximity but also at a cer-

tain distance there appear a great number of ameboid cells. The granular leucocytes are the earliest to come and during the first hours after injection they are found to be the most numerous. Their structure allows of an easy identification, their polymorphic nucleus being especially characteristic. Most of the leucocytes belong to the neutrophil class. Present in large numbers within the vessels, they are first to emigrate and to appear around the injected masses. Within the vessels and in close proximity to them they present the usual structure, but soon undergo considerable changes in their appearance. Their nuclei are often drawn lengthwise and appear in a thread-like shape. The chromatin in the nucleus is also arranged in the form of rod-like particles. This is of course due to intensive streaming movements exhibited by these cells. But a more substantial change takes place in their cytoplasm. This greatly increases in volume and though still granular is much less distinctly so.

In addition to the granular leucocytes, a number of ameboid cells with round nuclei appear soon. Twelve to fourteen hours after the injection they greatly increase in number and after twenty-four hours they are by far the most numerous among the cells infiltrating the region. These ameboid cells, which become most active in the process of digestion of the injected protein, exhibit the structure characteristic of the small lymphocytes. It is only after a thorough investigation that this conclusion has been reached. A few hours after injection when numerous polymorphonuclear leucocytes have already emigrated, the vessels throughout the tadpole and especially those in proximity to the injection begin to show an increasing number of small lymphocytes. The characteristic structure, which enables an easy recognition of these cells within the vessels, is also present even to minute details in cells which have recently emigrated and are situated near the injected substance. Small in size, they have a round nucleus containing numerous well-defined chromatin particles and no nucleoli. The nuclear membrane is chromatic and sharply defined. The cytoplasm is slightly basophilic and appears in the form of a narrow rim. Outside the vessels they exhibit numerous ameboid processes.

As these cells advance toward the injected mass they exhibit a series of changes, which make them rapidly acquire an entirely different structure and unroll a digestive activity little expected from the small lymphocytes. A rapid transformation of a small lymphocyte into a histiotopic wandering cell thereby takes place. Analogous changes have been observed in small lymphocytes, while in grafts of adult splenic tissue, the small lymphocytes wander out from the grafted tissue into the intercellular spaces of the allantois. Both nucleus and cytoplasm of these cells increase in size. The cytoplasm does not become more basophilic, as usually is the case with the hypertrophying mesenchymal cells. It stains light blue with Azur—II., and is often pervaded with minute vacuoles. The nucleus grows also; its chromatin, especially in the early stages, appears as in small lymphocytes in the form of irregular, rather heavy particles, but is soon converted into tiny fragments. The cells now differ entirely from the small lymphocytes in morphological appearance and wherever found in contact with edestin granules, rapidly ingest them.

The small lymphocytes are scarce in the circulation of a normal tadpole and the appearance of a great number of these cells, not only around, but in later stages within the injected mass, is at first glance rather difficult to account for. A study of the organs of the tadpole at this stage has revealed that the chief hemopoietic center is situated in the kidney. The blood-forming processes here exhibit a peculiarity not yet described in any hemopoietic organs of other embryos. The young blood stem cells, in the form of the lymphoid hemoblasts (large cell, basophilic cytoplasm, clear, round nucleus with a well-formed nucleolus) are here extremely scarce. Lymphoid cells are present in great numbers, but they exhibit the structure of small lymphocytes. The reciprocal relations of the blood cells in the kidney of the tadpole cannot be discussed at present, but it may be pointed out that the same characteristic feature was observed by one of us in the case of the axolotl years ago while studying the hemopoiesis in the perihepatic tissue in this animal.

The vessels in the kidney of the tadpole under experiment are always found to contain numerous small lymphocytes and an

intensive proliferation of these cells is observed in the hemopoietic tissue of this organ. No decrease of small lymphocytes is brought about by the excessive withdrawal of these elements, but, on the contrary, the lymphatic elements seem to become even more numerous and mitoses particularly frequent.

The shifting of numerous small lymphocytes to the injected region produces evidently a stimulating effect upon the hemopoietic tissue of the kidney, similarly to a bleeding of the animal. In this case however the white blood corpuscles only are carried away in greater numbers and those remaining proliferate more intensively. A proliferation of small lymphocytes in the lymphatic tissue has been found under various conditions, after administration of small doses of X-ray in particular. A direct stimulating effect upon the lymphatic tissue was attributed to the action of the X-rays. It is, however, questionable whether this stimulating effect might not prove to be a secondary phenomenon due either to destruction or to an intensive shifting of the small lymphocytes from the region of their origin.

A third category of wandering cells may be recognized around the injected material. They are mobilized mesenchymal cells, in the early stages very few in number and never numerous. They are large, have a rather basophilic cytoplasm, and their nuclei frequently exhibit nucleoli. They have been already mentioned and their first appearance was ascribed rather to the direct effect of the injury than to the presence of injected masses. At the end of the first day after injection a curious activity is observable in the mesenchymal cells which form under the basal membrane a practically uninterrupted layer. These cells, normally flattened against the surface of the basal membrane and in sections appearing fusiform in shape, are now seen to separate from the basal membrane and to protrude their processes in the direction of the injected material. They leave their original places and are found among other wandering cells. Easily recognizable at first they gradually undergo a series of changes in the same direction as described for the small lymphocytes, and at a later stage these cells are no longer recognizable as such.

In order to make a complete picture of the wandering cells in the region of the injection, eosinophilic leucocytes should be men-

tioned, but they are very scarce. An endothelial origin of some of the wandering cells cannot be excluded altogether since occasionally small vessels are injured and their endothelial cells might be transformed into wandering cells. Their number, however, must be of small account indeed in comparison with those brought in by the blood stream.

2. *Digestion of Injected Edestin.*—The process of digestion is inseparably connected in our mind with a specialized system, all of which is derived from the entoderm. This includes a special cavity, in which the process is completed outside of cells and tissues. A group of specifically differentiated secreting organs are furnishing the enzymes, which, though of utmost importance in digestion, still escape a more precise definition of their chemical structure. We still judge of the presence of enzymes by the result produced by them. Owing to the observation of results obtained in the unicellular organism, we easily accord to the protozoa an intracellular power of digestion. In this case the digestive ferments become active within a vacuole surrounded by living cytoplasm, and while the conditions within this vacuole bring about digestion of the ingested food (whether living or dead) they do not affect in the same manner the cytoplasm of the acting organism.

It was Metchnikoff's merit to have pointed out in the multicellular organism the rôle of phagocytosis with subsequent digestion of the ingested material. The phagocytes described by him belonged to two classes distinctly and differently organized, the polymorphonuclear leucocytes and the macrophages. The polymorphonuclear leucocytes did not require much interpretation, but the macrophages became an object of numerous investigations. Their nature and origin are much disputed, possibly because results obtained by study of definite cases and true, if limited to these, were extended beyond the sphere of their control. The macrophages are derived in turn from mesenchymal and from endothelial cells. Blood stem cells (lymphoid hemoblasts) were also seen to become macrophages. A special line of resting or histiotopic wandering cells, eminently phagocytic, was observed to develop from the mesenchyme in later embryonic stages. The

"cellules rhagiocrines" of Renaut and the clasmatocytes of Ranvier are endowed with phagocytic power. The polyblasts of Maximoff, derived from the small lymphocytes, are very active in inflammation processes.

No efforts have been spared to find structural peculiarities characterizing the mononuclear macrophages to the exclusion of lymphocytes. An ingenious method of identifying the macrophages was devised by H. Evans and elaborated by M. Simpson by staining a characteristic set of granules numerous in the mononuclear cells and very scarce in the lymphocytes.

That the class of macrophages, whether or not the mononuclear leucocyte of the blood stream belongs to it, are well-defined structural units, is not a disputed fact, nor is their phagocytic activity doubted. Their origin in the venous sinuses of the spleen, lymph nodes and bone marrow, in the region where endothelium and mesenchyme gradually merge into each other has been admitted. But do these well-established facts make the existence of a much more extended digestive activity in mesenchyme and its derivatives incredible in a multicellular organism? And why should digestive activity be necessarily limited to macrophages of serous cavities and to other cells identical with them as to their structure and origin?

A study of the conditions as they develop gradually in the embryonic organism will allow of an easy realization of the fact, that digestive activity is an inherent attribute of every cell, and that it is retained by that part of the mesoderm which remains the least differentiated in the form of mesenchyme. True that in vertebrates the ectodermal and mesodermal layers are soon separated from the yolk by the entoderm which remains in contact with the yolk and develops into a series of highly specialized digestive organs. But it is equally true that in earlier stages of development the cells of the primitive streak are in direct contact with the yolk and that the cytoplasm of all of them invariably contain yolk granules, which gradually disappear by intracellular digestion. All of the mesodermic phagocytes would derive their digestive power from the cells of the primitive streak, being their direct descendents. Actively manifested by the cells of the meso-

dermal anlage, the intracellular digestive power seems to be lost by a great number of highly differentiated mesodermic structures but is being retained by those mesenchymal elements, which, scattered through the whole organism, remain practically undifferentiated.

The conditions are by far more striking in those classes of animals whose eggs, as in the case of the tadpole, are rich in yolk, but segment completely. Though segmentation results in this case in uneven distribution of yolk among the various groups of cells, nevertheless all of them contain nutritive material and gradually digest it. The mesodermal cells are in this case developed in intimate association with the primitive entoderm, at a time part of it. The primitive blood cells are loaded with yolk granules gradually digested and used up. Later, however, the mesenchyme and its derivatives not being in contact with undigested protein, digestive capacity is not exercised by these tissues. But phagocytic digestive power remains inherent in them and is revealed every time mesenchyme or its derivatives are in the presence of particulate protein.

As seen from the preceding section, the injected mass is surrounded, six hours after injection, by a great number of wandering cells (Fig. 1) of different nature. Some of them are actually seen within the injected mass. The polymorphonuclear leucocytes are not only first to appear around the injected edestin, but they also are the first to bore their way into the rather compact mass of edestin. Wherever a group of edestin particles has been carried by the injection deeper into the tissues and found detached from the more compact mass, the granular leucocytes gather around them and often at this early stage edestin particles are found already ingested by them. While actively ingesting large amounts of edestin particles, the granular leucocytes may attain a considerable size. Their cytoplasm is often seen to be reduced to a narrow rim holding a large amount of ingested material. Its granular structure, becoming less distinctive, while the cell is moving toward the injected mass, has now practically disappeared altogether. But the nucleus retains its polymorphous structure and this makes the identification of these cells always easy. It is remarkable that a great number of polymorphonuclear leucocytes,

found in the midst of the injected mass, are manifesting evident signs of degeneration. A chromatolysis is frequently observed in their nuclei at an early stage and later on these cells, even while containing in their cytoplasm edestin granules, are themselves subject to phagocytosis by other cells.

By far the most important rôle in the actual digestion of the injected mass belongs to those cells which are brought in by the blood stream in the form of small lymphocytes. We do not mean to say that the small lymphocytes as such devour a great amount of edestin and digest it. But there is no doubt that cells, the structure of which is identical to that of the small lymphocytes, emigrate from the blood vessels, approach the injected mass and, while approaching it, promptly change their morphological features. Such cells, lymphoid phagocytes as we will call them, if in contact with the injected mass of edestin, are seen, at the stage of six hours after injection, to contain edestin particles in their cytoplasm. The surface of their cytoplasm becomes indented and tiny cytoplasmic processes are seen to protrude between the injected edestin particles, to entirely surround them, to incorporate them into larger vacuoles and to repeat the same process again and again. Sometimes larger groups of edestin particles are seen to be surrounded simultaneously by larger cytoplasmic processes and incorporated into a common vacuole.

A greater and greater number of cells invade the edestin mass until a stage is reached, about 24 hours after injection, in which all of the edestin granules are found within the cells. At this time (Fig. 2) the conditions within the injected mass are difficult to analyze. The finest celloidin sections give rather obscure pictures. All the edestin granules are found in larger or smaller spaces surrounded by cytoplasmic strands, but the boundaries of the individual cells are indistinct. Cytoplasm, a little more abundant around the nucleus and easily recognizable as a part of a definite cell, is seen to merge gradually into cytoplasmic strands of neighboring cells. The whole region appears in the form of vast plasmodia, here and there interrupted by more or less definite fissures (Fig. 2).

Figure 3 shows the development of these conditions in the peripheral part of the injected mass. A vessel containing ery-

throcytes and a number of small lymphocytes is found to be in close apposition to it. A group of small lymphocytes in its proximity are seen with large ameboid processes. At this stage most of the edestin granules are incorporated in the cytoplasm of the lymphoid phagocytes, only a few groups still remaining outside. Most of them are situated in larger or smaller vacuoles. Apparently one cell may possess a number of such vacuoles. Though in general the cells appear to be well separated from one another, there may also be observed groups of cells which seem to have flown together.

As early as 16–24 hours after injection a change takes place in the numerical relation between the polymorphonuclear leucocytes and the lymphoid phagocytes, now both appearing in the form of large cells with vast vacuoles containing large amounts of edestin granules. The polymorphonuclear leucocytes, even more abundant than the lymphoid phagocytes early after the injection become now scarce and rather difficult to identify. A granular leucocyte, as admitted, is a highly specialized cell, stable and no longer capable of developmental changes. Would such a cell under experimental conditions be still capable of modifying its metabolism so as to manifest a fundamental change in its structure? This idea, incompatible as it seems with our knowledge of histogenesis of blood cells, is forced upon one's mind by the rapidity of the change in the numerical relation of these two different kinds of phagocytes. And only a detailed analysis of the conditions obtaining within the injected mass permits of a final refutation of this idea. The polymorphonuclear leucocytes numerous in early stages become less and less so, because of the association of various factors all working in the same direction. Soon after the first wave of emigration brings around the injected mass a large group of polymorphonuclear leucocytes, lymphocytic cells become more and more abundant within the blood stream and are seen to emigrate in larger and larger numbers. At the same time the polymorphonuclear leucocytes, well within the edestin mass, begin to degenerate in great numbers. In later stages many of them are seen to be ingested by the lymphoid phagocytes. As

a result of the simultaneous association of these conditions, 1 or 2 days after injection almost all of the cells in the area of injection are represented by lymphoid phagocytes.

There is no doubt that while the process of ingesting the edestin takes place, actual digestion begins at about the same time. The digestion in this case is intracellular and similar to that observed in the protozoa. The edestin particles, found in rather large vacuoles, are gradually disappearing, and, on the basis of this observation, the conclusion must be reached, that enzymes are liberated by these cells into vacuoles in conditions permitting of digestion of the protein particles and not affecting the cytoplasm surrounding the vacuole. A rather interesting detail is observed in the digestive activity of the phagocyte. The edestin granules are frequently seen to be surrounded individually by thin strands of cytoplasm. Early after injection, the phagocytes are invariably seen to contain large groups of edestin granules in single vacuoles, but in later stages these groups are seen to be gradually separated by cytoplasmic strands (Fig. 4, four days) until every granule is surrounded separately. Whether, however, this step is necessary is difficult to tell.

While ingestion of edestin by the phagocytes has resulted in the formation of vast indivisible plasmodia (Fig. 2), the digestion and resorption of the edestin leads again to a definition of separate cell units. Two days after injection the region appears very similar to the condition found in the periphery of the injected mass at an early stage (Fig. 3). The phagocytes appear again individually separated and in denser regions only a few plasmodia are observed. This separation is completed 3 or 4 days after injection, as Fig. 4 shows. At this time (4 days after injection) the digestion in most of the cells has been completed. Some of them still show a large vacuole containing a number of edestin granules, but such cells have become scarcer. Other phagocytes contain two or three small vacuoles with a limited number or even with single granules. Cells are observed, also, which do not contain more than 2 or 3 small granules and finally a number of cells are found without any. Digestion in these cells has been apparently completed.

The digestive power is being exercised by the lymphoid phagocytes in a most conspicuous way. It is questionable, however, whether or not polymorphonuclear leucocytes actually digest the edestin particles found rather numerous in their cytoplasm at earlier stages. Mention was made above regarding degenerative changes frequently observed in the leucocytes within the injected mass. But even those leucocytes which persist at a stage when a great part of the edestin has been digested do not seem to prosper. They are now frequently seen ingested by the lymphoid phagocytes and undergoing a digestion within them. In Fig. 4 three lymphoid phagocytes are seen to contain polymorphonuclear leucocytes in their cytoplasm; the latter, themselves, had acted as phagocytes, for numerous edestin granules are still present in their cytoplasm. They succumb, however, now to the phagocytic activity of lymphoid phagocytes.

The structure of the lymphoid phagocytes is so characteristic as to permit an unmistakable judgment regarding their past activity and origin. And the new phase of their digestive activity which now is directed against the polymorphonuclear leucocytes is of great interest. Both lymphoid phagocytes and polymorphonuclear leucocytes develop in the same hemopoietic center and from the same stem cells. Both were brought in by the blood current to the region of the injection; they both moved into the injected mass and began their active ingestion of the edestin particles. But while ingestion by lymphoid phagocytes is followed by an intensive digestive activity, the polymorphonuclear leucocytes, though containing ingested edestin, seem to remain inert. The exercise of digestive activity by the lymphoid phagocytes seem to have stimulated and sharpened their digestive power and they begin to display it against cells of their own kind, the granular leucocytes.

The digestion of the injected mass of edestin is completed in about 6 or 7 days. There is no more trace of edestin granules in the tadpole tail at this time and the whole region, in which the edestin appeared as a compact strand of a 0.3–0.5 mm. in thickness, has been reduced to a line hardly perceptible to the naked eye and no more than 100 μ thick. Not only has the whole mass

of injected edestin disappeared from this region, but the millions of cells which after 2 days of injection invaded it have left it. There is no indication whatever of degenerative processes in the lymphoid phagocytes. Their changes and fate will be discussed in the following paragraph.

3. *Phagocytes after Digestion.*—A cursory glance and comparison of a small lymphocyte traversing within the blood current the region of injection (Figs. 4 and 5) with the phagocytes scattered through it, is sufficient to establish the difference between these two types of cells, as such. Nevertheless, as seen from the preceding sections, the phagocytes did develop from the small lymphocytes of the blood stream through a series of changes which the emigrated cells exhibited while approaching the injected mass. The exercise of digestive activity by the lymphoid phagocytes seems to have further fixed the acquired structural characters and most of the phagocytes are seen to retain their new structure. They now appear in the form of large cells with especially abundant cytoplasm. They are very similar to the usual types of phagocytes encountered in the loose connective tissue and described under many different names (resting or histiotopic wandering cells, clasmatocytes, macrophages, cellules rhagiocrines). Only the loose mesenchyme in the tadpole does not normally contain any wandering cells. The mesenchymal cells though observed to transform occasionally into wandering cells after injection, are extremely scarce. No mononuclear leucocytes are present in the blood current at that time. The only cells capable of further progressive development and differentiation in the tadpole blood are cells which exhibit the structure of small lymphocytes. They are seen indeed to give rise suddenly to numerous generations of phagocytes. These cells after digestion retain their newly acquired structure and are easily recognizable. They exercise their digestive power locally but do not remain in this region indefinitely. They are seen to increase in number while supplies of injected edestin last, but as soon as all of it is consumed, the immigration of new cells stops. The phagocytes remain in this region while they are actively digesting the injected edestin, but then begin to emigrate individually. By far the great-

est part of the phagocytes are seen to leave the region and settle in new places.

The phagocytes are seen to be very active in leaving the region of the injection as soon as they have completed the digestion of the ingested particles. The large aggregations of these cells are being dispersed, and 4 days after injection the egress of phagocytes is very intensive. At this time lymphoid phagocytes can be detected at a good distance from their previous location. Small groups of phagocytes are gathered directly under the epidermis. Two or three weeks after injection they can still be recognized as cell units with definite structure. What is even more remarkable is, that the cells are seen not infrequently to undergo mitosis.

Though on the basis of the present experiments a complete description of the distribution of the phagocytes cannot be given, there are undoubtful indications that their new whereabouts are not confined to the tadpole tail. The phagocytes, while moving from the region of injection, come not infrequently across vessels. Vessels have grown by this time also into the injected region, and numerous phagocytes are now seen to be directly applied against the thin-walled vessels. It is also possible to detect some of these phagocytes within the vessels. It is natural that such pictures are extremely rare, because the phagocytes, once entered into a vessel, are promptly carried away.

The uninterrupted egress of the phagocytes reduces the wide strands of densely accumulated phagocytes to a hardly visible scar (Fig. 5). The cells in it are of two kinds: Typical mesenchymal cells and typical histiotopic or resting wandering cells. The latter are the less numerous. As compared with Figure 4, Figure 5 shows that the egress of the phagocytes is chiefly responsible for the loosening of the tissue and for the shrinking of the previously wide strand of the densely infiltrated region.

Conclusions.

The introduction of a large amount of edestin into the mesenchymal plate of the tail of the tadpole produces a local and a general reaction in the organism. The local reaction consists in a response of mobile and mobilisable cells and results in a dense infiltration of the injected mass with consecutive ingestion and

digestion of the injected mass. The injected suspension of edestin is a powerful chemotactic agent for both the granular leucocytes and small lymphocytic cells.

Secondarily a general reaction appears in the blood-forming tissue of the kidney. It consists in intensive proliferative processes which seem to be in relation with the egress of small lymphocytes from this tissue.

The lymphocytic cells emigrated from the vessels gather around the injected mass, hypertrophy and gradually transform into typical histiotopic wandering cells or lymphoid phagocytes. They ingest large amounts of edestin granules and exercise a digestive capacity. The digestive activity manifested by these cells seems both to stabilize the newly acquired structure of the phagocytes and to sharpen further their digestive power.

The granular leucocytes though active in ingesting the edestin granules do not seem to be capable of digesting them. These cells, especially those containing edestin particles in their cytoplasm, are seen to succumb finally to the digestive activity of the lymphatic phagocytes. There exist undoubtful indications that some of the phagocytes have derived from the mesenchymal cells, but their rôle cannot be easily determined in these experiments.

Two days after injection all the edestin granules are found within the phagocytes; seven days after injection all of the edestin has disappeared.

The digestive processes within the injected region not only result in the formation of a generation of new eminently phagocytic cells, but seem to reflect secondarily upon the whole organism. Innumerable cells of the type of small lymphocytes are not only transformed into lymphoid phagocytes, but these phagocytes are soon found at a considerable distance from the region of injection and part of them are probably distributed through the blood current to more distant parts of the organism.

BIBLIOGRAPHY.

1. **Clark, E. R.**

'16 A Study of the Reaction of Mesenchyme Cells in the Tadpole's Tail toward Injected Oil Globules. Anat. Rec., Vol. 11, No. 1, August.

2. **Clark, E. R. and Clark, E. L.**
'17 A Study of the Relation of Lymphatic Endothelium and of Leucocytes, in the Tadpole's Tail, toward Injected Fat. Amer. Jour. Anat., Vol. 21, No. 3.

3. **Clark, E. R. and Clark, E. L.**
'18 Phagocytosis of Carbon and Carmine Granules in the Transparent Tails of Tadpoles. Anat. Rec., Vol. 15, No. 3.

4. **Clark, E. R. and Clark, E. L.**
'20 Reactions of Cells in the Tail of Amphibian Larvæ to Injected Croton Oil (Aseptic Inflammation). Amer. Jour. Anat., Vol. 27, No. 2.

5. **Clark, E. R. and Clark, E. L.**
'21 The Reaction of Living Cells in the Tadpole's Tail toward Injected Starch Granules. Amer. Asoc. Anatomists, Abstracts of Papers.

6. **Danchakoff, V.**
'18 Equivalence of Different Hematopoietic Anlages. (By method of stimulation of their stemcells.) II. Grafts of Adult Spleen on the Allantois and Response of the Allantoic Tisues. Amer. Jour. Anat., Vol. 24, No. 2.

7. **Danchakoff, V.**
'21 Digestive Activity of Mesenchyme. *A*. The Ehrlich Sarcoma Cells as Object. Amer. Jour. Anat., Vol. 29, No. 4.

8. **Danchakoff, V.**
'21. Digestion of different proteins by the mesenchyme and its derivatives in the tadpole. Proc. of the Am. Ass. of Anat. The Anat. Record, Vol. 21, Apr.

9. **Evans, H.**
'15 The Macrophages of Mammals. Amer. Jour. Phys., Vol. 37.

10. **Maximoff, A.**
'02 Experimentelle Untersuchungen über die entzundliche neubildung von Bindegewebe. Zeigler's Beiträge.

11. **Metchnikoff, E.**
'01 L'immunite dans les maladies infectieuses.

12. **Murphy, J. B. and Nakahara, W.**
'20 The Lymphocyte in Natural and Induced Resistance to Cancer. V. Histological Study of the Lymphoid Tissue of Mice with Induced Immunity to Transplanted Cancer. Jour. Exper. Med., Vol. 31, p. 1.

13. **Simpson, Miriam E.**
'21 Vital Staining of Human and Mammalian Blood, with Special Reference to the Separation of the Monocytes. Amer. Assoc. Anatomists, Abstracts of Papers.

EXPLANATION OF FIGURES.

All the figures are camera lucida drawings made with the Zeiss apochromat oil immersion 2 mm., and with the compensatory ocular 8.

PLATE I.

FIG. 1. Zone of injection 6 hours after experiment. Edge of the injected strand of edestin at the right side of the figure. A vessel at the left contains erythrocytes, numerous small lymphocytes and one neutrophil polymorphonuclear leucocyte. The emigrated small lymphocytes in the proximity of the vessel are small. They hypertrophy while approaching the injected edestin. A number of granular leucocytes and three lymphoid phagocytes have penetrated the mass of edestin and begin to ingest its particles.

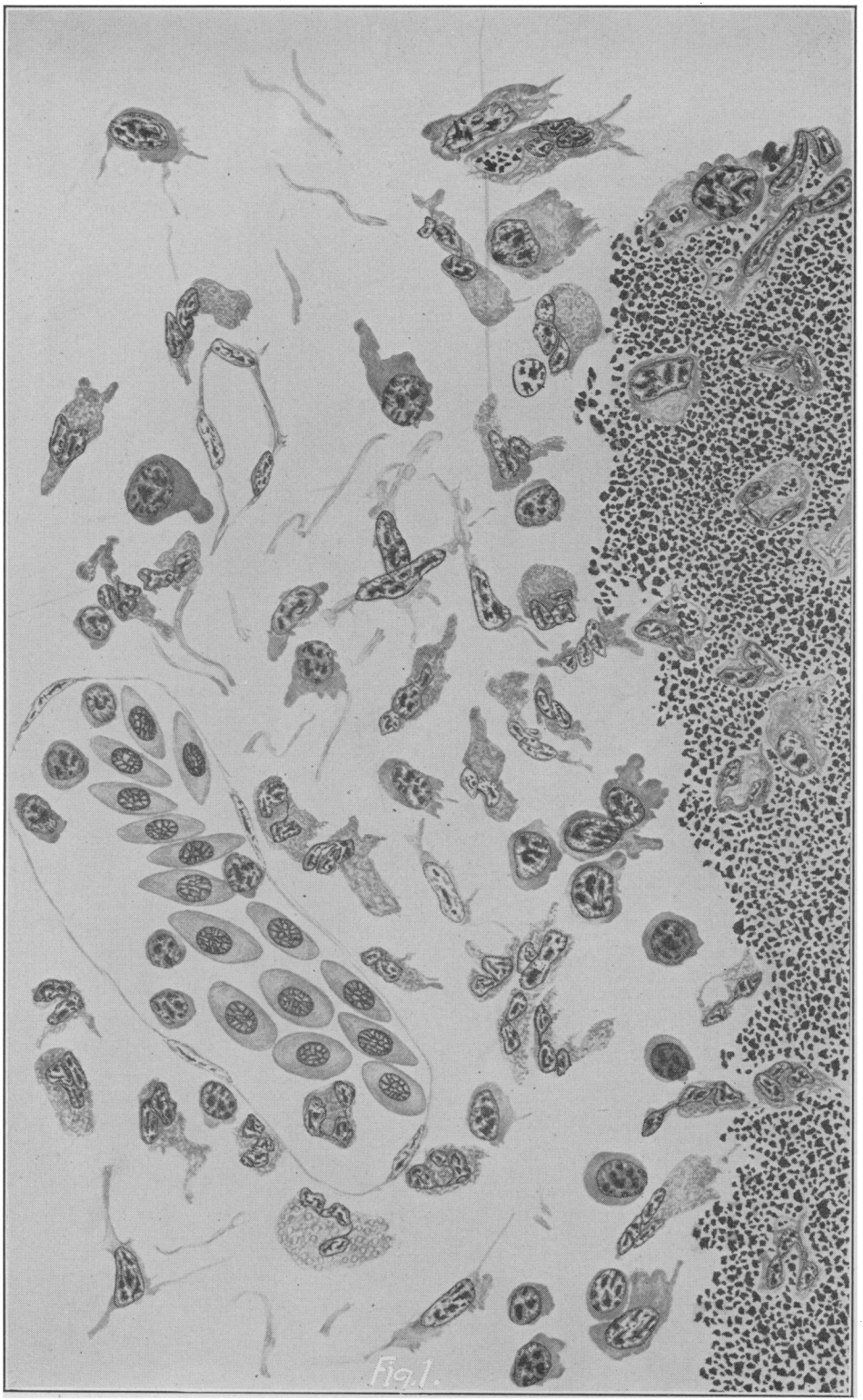
Fig. 1.

PLATE II.

FIG. 2. Zone of injection 24 hours after operation. Central part of the large strand of ingested edestin. The cells form a uniform plasmodium. The edestin granules are seen to be separated into groups and surrounded by thin cytoplasmic processes.

FIG. 3. Zone of injection 24 hours after operation. Edge of the large strand of injected edestin. A vessel is seen to contain erythrocytes, small lymphocytes and a granular leucocyte. Most of the phagocytes contain large amounts of edestin and appear as separate cells, though a few of them have flown together.

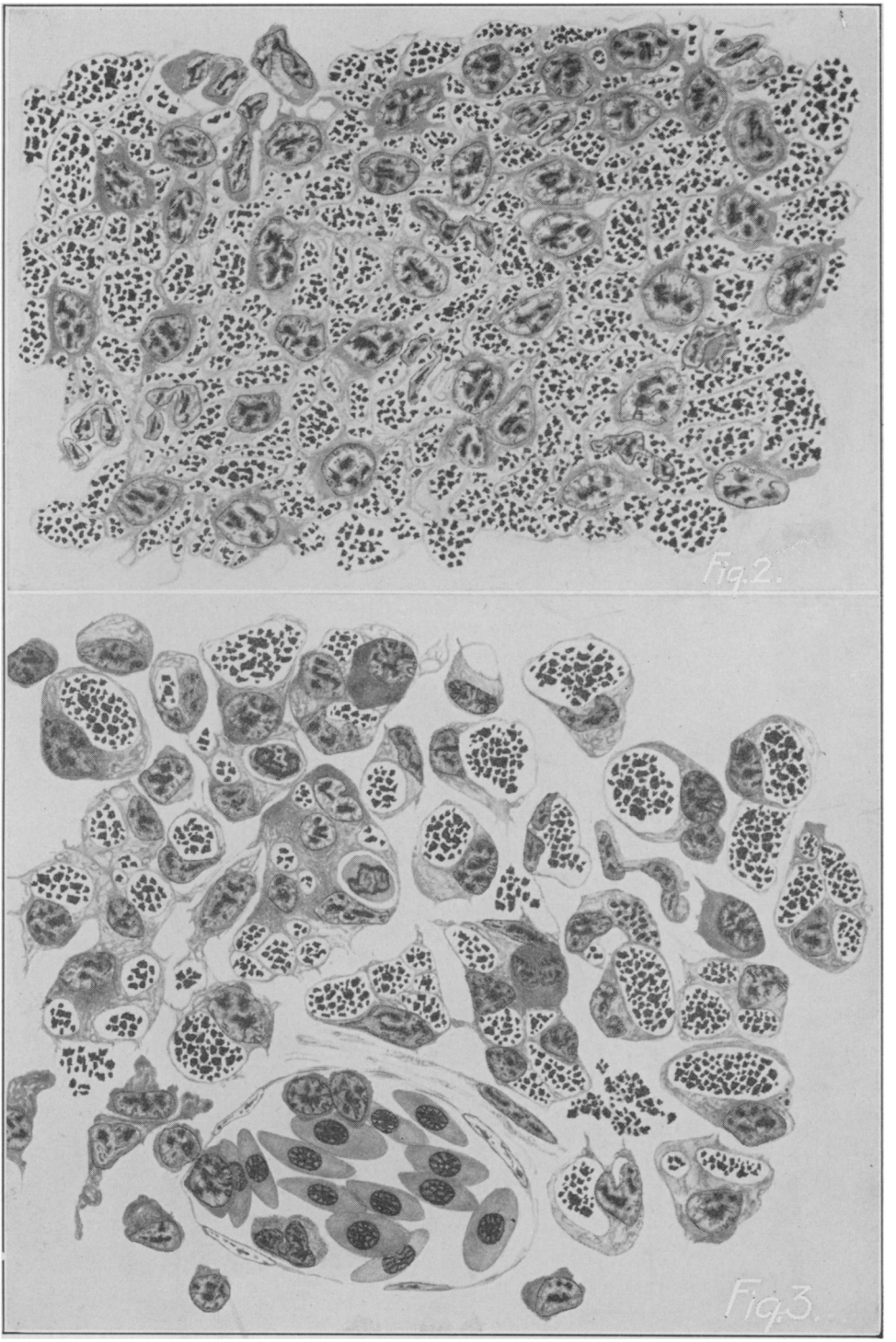
Fig. 2.
Fig. 3.

PLATE III.

FIG. 4. Zone of injection 4 days after experiment. A great number of phagocytes have completed the digestion of the ingested edestin; others contain still either small groups or even single particles of edestin. Three granular leucocytes with edestin in their cytoplasm are seen to have been ingested by the lymphoid phagocytes. The phagocytes become much less numerous than in the preceding stage.

FIG. 5. Zone of injection 7 days after experiment. A thin scar remains after completion of digestion of the injected edestin. It consists of mesenchymal cells, scarce histiotopic wandering cells (lymphoid phagocytes) and a few vessels traversing the zone.

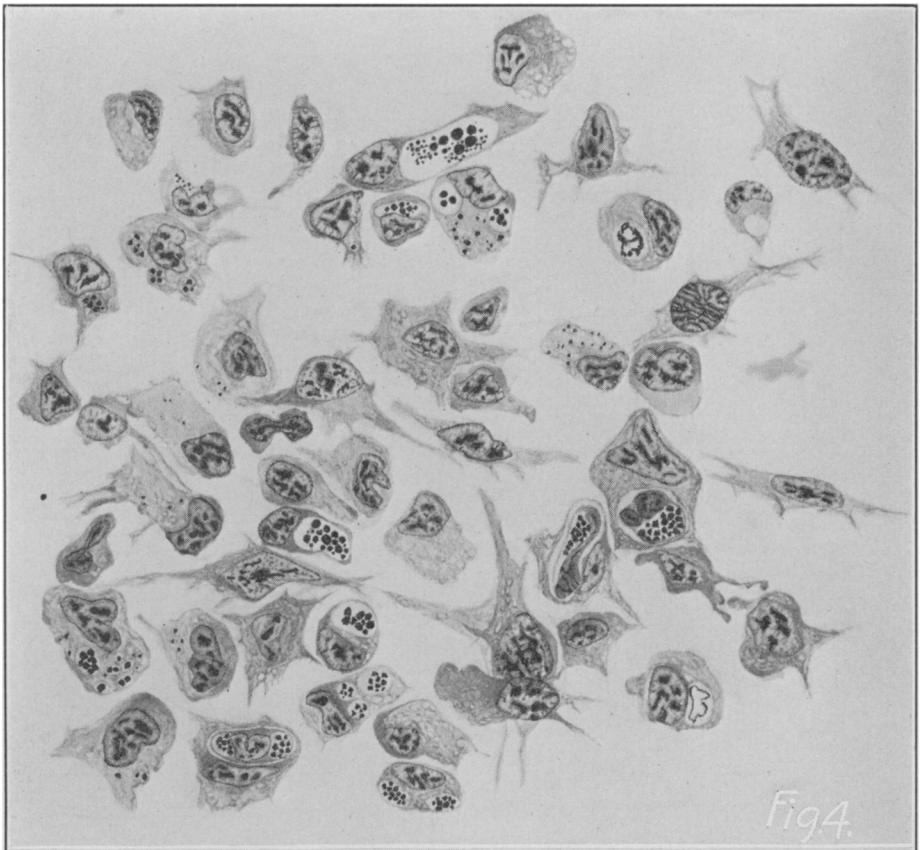
Fig.4.

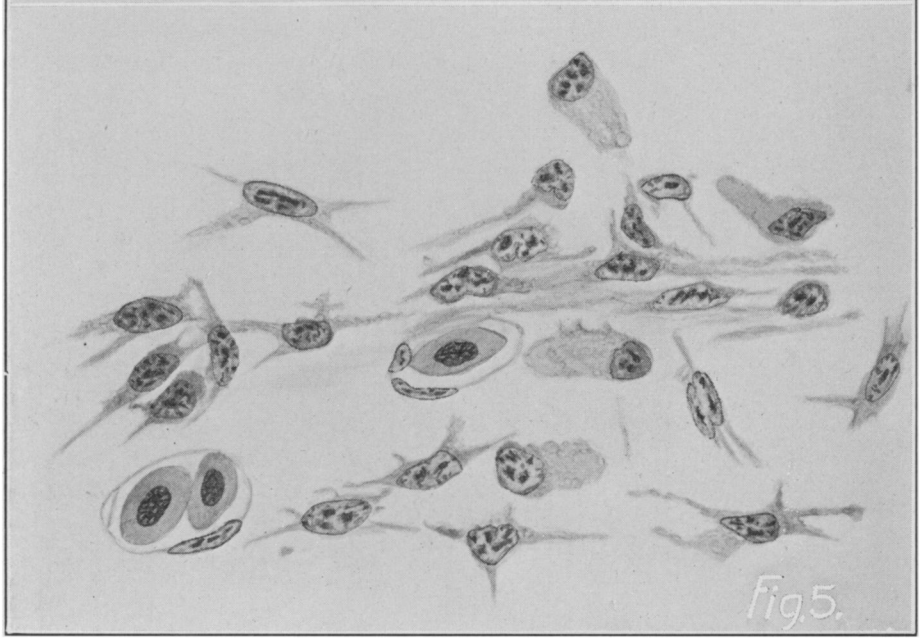
Fig.5.